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Towards the synthesis of light stable coenzyme B_{12} analogs

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Abstract

The organometallic complex coenzyme B_{12} (adenosyl cobalamin, AdoCbl) is not only an essential coenzyme in many biochemical reactions of most if not all living organisms but has lately been shown to play a crucial role in the gene-regulation of B_{12} related genes. As a consequence, coenzyme B_{12} has been a target of intense research. However, investigations of AdoCbl have often been hampered due to its high light-sensitivity leading to decomposition of the compound within a few seconds. Here, we describe a strategy to synthesize more light stable coenzyme B_{12} analogs, which show similar steric properties as adenosyl cobalamin. The synthesis, structural characterization as well as the pH dependent "base-on/base-off" behavior of cyanide bridged vitamin B_{12} conjugates with either a *cis*-$[(NH_3)_2Pt]^{2+}$ or an $[enPt]^{2+}$ moiety, leading to *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^{+}$ (**1**) and $[enPtCl\text{-}vitB_{12}]^{+}$ (**2**) are reported. Subsequent reaction of *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^{+}$ with the model nucleobase 9-methyladenine leads to the corresponding adduct, where the adenine moiety is coordinated to the Pt^{2+} center either *via* N1 or N7. This compound is light stable and harbors the adenine moiety in the same distance of 5 Å above the corrin plane as present in the highly light-sensitive adenosyl cobalamin.

Keywords: adenosyl cobalamin, B_{12}, *Cisplatin*, light-sensitivity

1. Introduction

Coenzyme B_{12} (AdoCbl) is the structurally most complex of all known vitamins and is essential for most or even all living organisms as a cofactor for numerous enzymatic reactions in the metabolic pathways [1]. Only recently it was discovered, that coenzyme B_{12} does not only interact with proteins but can also bind specifically and with high affinity to conserved RNA sequences found in the 5'-untranslated region of mRNA [2]. This high affinity binding between a small molecule and its target RNA sequence, the so-called riboswitch, exhibits an alternative pathway for the regulation of gene-expression [3-6]. Binding is highly specific and the adenine moiety of adenosyl cobalamin seems to play an important role in recognition of the coenzyme by the RNA. In the case of the two best studied B_{12}-responding riboswitches, the so-called *btuB*-leading sequence of *Escherichia coli* and *Salmonella typhimurium* (encoding for a B_{12}-transport protein) and the leading-sequence of the *cob*-operon of *S. typhimurium* (encoding for different proteins of the B_{12} biosynthetic pathway) coenzyme B_{12} is the derivative contributing to gene regulation [7-9]: Binding of B_{12} derivatives is rather tight with dissociation constants of around 0.3 μM (AdoCbl), 3 μM (MeCbl) and 30 μM for vitamin B_{12}, while aquo-cobalamin has no effect on the gene regulation [7]. However, coenzyme B_{12} is known to be highly light-sensitive and to decompose within a few seconds [10]. Due to this fact, coenzyme B_{12} is difficult to handle in many biochemical applications and experiments such as the research on the B_{12}-responding riboswitches [8]. In the following, we describe our efforts towards the synthesis of more light-stable AdoCbl derivatives while preserving the crucial structural features of this complex.

Recently, a method was described by Mundwiler et al. to derivatize the cyanide ligand of vitB_{12} by coordinating a *cis*-$[(NH_3)_2PtCl]^+$ unit to this ligand and subsequently to attach a guanine moiety to the Pt^{2+} center [11]. Comparison of the crystal structures of the *cis*-$[(NH_3)_2Pt(9\text{-MeGua})\text{-vit}B_{12}]^{2+}$ (9-MeGua = 9-methylguanine) adduct [11] and of 5'-desoxyadenosylcobalamin [12] show close structural similarities. The Co-Pt distance of

around 5 Å found in the adduct compares well with the Co-C1' distance of 5.05 Å in 5'-desoxyadenosylcobalamin, as do the orientations of the nucleobases, positioned parallel to the corrin ring. This finding opens the possibility to synthesize more light stable AdoCbl analogs by coordinating an adenine moiety to the Pt^{2+} center of such cyano-bridged vitB_{12} derivatives.

Thus, in the present context we are focusing on the preparation and characterization of *cis*-$[(NH_3)_2PtCl]^+$ [11] and newly also the $[enPtCl]^+$ adducts of vitamin B_{12} and their subsequent reaction with the model nucleobase 9-methyladenine. In the re-determined crystal structure of the *Cisplatin* adduct all anions could be localized, and this structure will be compared in the following to the new structure of the $[enPtCl]^+$ adduct of vitB_{12}. We further investigated the "base-on/base-off" behavior of the two complexes and compared it with vitamin B_{12} as well as with coenzyme B_{12}. Reaction of *cis*-$[(NH_3)_2PtCl\text{-vit}B_{12}]^+$ with 9-methyladenine leads to two adenine adducts, in which the nucleobase is coordinated to the Pt^{2+} center either through its N1 or N7 position. This model compound is light-stable, but compares structurally very well to adenosyl cobalamin with respect to the position of the adenine base.

2. Experimental Section

2.1 Starting Materials

9-Methyladenine [13,14], *cis*-$(NH_3)_2PtCl_2$ [15,16] and *cis*-$[(NH_3)_2PtCl\text{-vit}B_{12}]CF_3CO_2$ (**1**) [11] were synthesized according to the literature. Chemicals used were purchased from Calbiochem, San Diego, USA (vitamin B_{12} (99.6%)), Alfa Aesar, Karlsruhe, Germany (dichloro(ethylenediamine)platinum(II) (59.82% Pt)), Fluka, Buchs, Switzerland or ACROS Organics, Geel, Belgium, and were used without further purification.

2.2 Instrumentation

HPLC analyses were performed on a Hitachi Elite LaChrom system equipped with a L-2400 UV detector and a Nucleodur C18 Gravity RP column (5 µm particle size, 110 Å pore

size, 250x4 mm, Macherey-Nagel). The HPLC-solvents used were 0.1% trifluoro acetic acid in H_2O (A) and methanol (B). The preparative HPLC separation was performed on a Varian Prostar system equipped with two Prostar 215 pumps, a Prostar 320 UV/Vis detector and a Nucleosil C-18ec RP column (7 μm particle size, 100 Å pore size, 250x21 mm, 14 mL min^{-1} flow rate, or 250x40 mm, 40 mL min^{-1} flow rate, Macherey-Nagel). ESI mass spectra were measured on a Waters Q-TOF Ultima API spectrometer, while the LC-MS was performed using ESI detection on a AQUA Thermo Finnigan spectrometer and a Sorbax C-18 column (3 μM pore size, 150x21 mm). UV/Vis spectra were recorded at 25 °C on a Varian Cary 500 spectrometer. IR spectra were recorded on a Perkin-Elmer Spectrum BX spectrometer in KBr pellets. All NMR spectra were recorded at 298 K with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as a reference; ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AV 700 MHz spectrometer with a CP-TXI cryoprobe, [^{1}H,^{1}H]-COSY and [^{13}C,^{1}H]-HSQC measurements on a Bruker AV 600 MHz spectrometer equipped with a CP-TCI triple resonance cryoprobe and ^{31}P-NMR and ^{195}Pt-NMR spectra on a Bruker DRX 500 MHz spectrometer with a 5 mm BBO probehead.

2.3 Preparations

[enPtCl-vitB_{12}]CF_3CO_2 · 15.8 H_2O · 1.1 $(CH_3)_2CO$ (**2**): A solution of $enPtCl_2$ (72 mg, 0.22 mmol) and $AgNO_3$ (37 mg, 0.22 mmol) in H_2O (8 mL) was stirred under N_2 atmosphere and in the dark for 2.5 h at 35 °C. The precipitated AgCl was removed by centrifugation (10 min, 6000 rpm) and washed with H_2O (4 mL). Vitamin B_{12} (300 mg, 0.22 mmol) was added to the combined solutions of $[enPtCl(H_2O)]^+$ (12 mL) and stirred under N_2 atmosphere and exclusion of light for 17.5 h at 50 °C. The reaction was followed by analytical HPLC on a reversed phase column (gradient: 5 min at 75% 0.1% trifluoro acetic acid and 25% methanol, then linear to 100% methanol within 24.5 min, 0.5 mL min^{-1} flow rate). The solvent was removed *in vacuo*, and the crude product purified by preparative HPLC on a C-18 reversed

phase column (gradient: linear from 100% A to 20% B in 5 min, linear to 65% B in 40 min and then linear to 100% B within 5 min). The peak from 18 to 23 min was collected, the solvent removed *in vacuo* and the red solid dissolved in ca. 3 mL H_2O. Lyophilization of this product fraction gave 340 mg of **2** as a purple powder (0.196 mmol, 89%). Recrystallization by vapor diffusion of acetone into an aqueous solution yielded the complex with $CF_3CO_2^-$ as anion, as well as 1.1 acetone and 15.8 water molecules per asymmetric unit. ^{1}H NMR (D_2O, 600 MHz): δ = 7.28 (s, 1H, H-B7), 7.07 (s, 1H, H-B2), 6.50 (s, 1H, H-B4), 6.35 (d, J = 2.8 Hz, 1H, H-R1), 6.08 (s, 1H, H-10), 4.71 (ddd, J = 8.5, 8.5, 4.2 Hz, H-R3), 4.35-4.27 (m, 3H, H-3, H-176, H-R2), 4.10 (d, J = 10.4 Hz, 1H, H-19), 4.05 (d, J = 3.9 Hz, 1H, H-R4), 3.92 (d, J = 12.0 Hz, 1H, H_a-R5), 3.75 (dd, J = 12.8, 3.8 Hz, 1H, H_b-R5), 3.61 (d, J = 14.2 Hz, 1H, H-175), 3.53 (dd, J = 11.0, 5.3 Hz, 1H, H-8), 3.35 (d, J = 10.2 Hz, 1H, H-13), all other peaks were not specifically assigned. ^{13}C NMR (D_2O, 150.94 MHz) δ = 180.4 (C-4), 179.1 (C-16), 177.8, 177.5, 176.8, 176.7, 175.6, 175.3, 174.6, 174.5, 173.8 (9C, C-9, C-11, C-22, C-33, C-72, C-83, C-133, C-173, C-182), 165.8 (C-14), 165.3 (C-6), 141.5 (C-B2), 136.2 (C-B9), 135.0, 133.0 (2C, C-B5, C-B6), 129.5 (C-B8), 116.0 (C-B4), 111.4 (C-B7), 107.2 (C-5), 103.8 (C-15), 94.6 (C-10), 86.9 (C-R1), 84.9 (C-1), 81.8 (d, J = 32.7 Hz, C- R4), 74.9 (C-19), 72.8 (d, J = 13.3 Hz, C-R2), 72.6 (d, J = 25.2 Hz, C-R3), 68.5 (t, J = 25.3 Hz, C-176), 60.1 (C-R5), 59.1 (C-17), 56.0 (C-3), 55.1 (C-8), 53.4 (C-13), 50.9 (C-7), 47.99, 47.97, 47.96 (3C, C-12, $C11_{en}$, $C12_{en}$), 46.97 (C-2), 45.0 (q, J = 16.0, C-175), 43.3, 43.0 (2C, C-21, C-71), 38.8 (C-18), 34.6, 34.2, 32.4, 32.1, 31.3, 31.23, 31.20, 27.7, 25.8, 25.7 (10C, C-12B,C-31, C-32, C-81, C-82, C-131, C-132, C-171, C-172, C-181), 19.67, 19.00, 18.95, 18.83, 18.76, 18.74, 16.46, 16.10, 15.27, 14.88 (10C, C-B10, C-B11, C-1A, C-2A, C-7A, C-12A, C-17B, C-51, C-151, C-177. ^{31}P-NMR (D_2O, 202.5 MHz): δ = –0.701 ppm. ^{195}Pt-NMR (D_2O, 107.5 MHz): δ = –2700 ppm. ESI-MS: m/z: 823.26 $[M]^{2+}$. IR (KBr): ν = 2195 cm^{-1} (CN). UV/Vis (H_2O): $\lambda(\log\varepsilon)$ = 250.0 (4.1), 279.0 (3.8), 362.0 (4.05), 515.0 (3.5), 550.0 (3.5 mol L^{-1} cm^{-1}).

cis-$[(NH_3)_2Pt(9\text{-MeAde})\text{-vitB}_{12}](CF_3CO_2)_2$ (**3**): A suspension of **1** (20 mg, 11.7 µmol) and

9-methyladenine (88 mg, 0.59 mmol) in H_2O (1 mL) was stirred under N_2 atmosphere and exclusion of light for 1 week at 45 °C. Analytical HPLC on a reversed phase column (gradient: 5 min at 75% 0.1% trifluoro acetic acid and 25% methanol, then linear to 100% methanol within 24.5 min, 0.5 mL min^{-1} flow rate) showed the formation of two new peaks at 5.6 and 7 min next to the peaks of the reagent and of vitamin B_{12} deriving from decomposition. The solvent was removed *in vacuo*, and the crude product purified by preparative HPLC on a C-18 reversed phase column (gradient: linear from 100% A to 20% B in 5 min, linear to 65% B in 40 min and then linear to 100% B within 5 min). Only a small fraction (at 21.6-23 min) of a broad peak was collected. Lyophilization of the product fraction gave **3** in 49% yield (11 mg, 5.8 μmol, yield calculated based on two $CF_3CO_2^-$ anions) as a mixture of two isomers (N1 and N7 coordinated to the Pt^{2+} center), which could not be separated so far. ^{1}H NMR (D_2O, 700 MHz, only resonances of the coordinated 9-MeAde are given): δ = 8.37, 8.36 (2 s, 1H for each isomer, Ade-H2/H8, see also text), 8.22, 8.20 (2s, 1H for each isomer, Ade-H2/H8, see also text), 3.85, 3.83 (2 s, 3 H for each isomer, Ade-C9H_3). ^{195}Pt-NMR (D_2O, 107.5 MHz): δ = –2573.5 ppm. LC-MS: m/z: 865.1$[M-4H]^{2+}$.

2.4 X-ray crystallography

Crystal data for compounds **1** and **2** were collected on a STOE IPDS system using graphite monochromated Mo K_α radiation (λ = 0.7107 Å) and a low-temperature device at 183 K. For data reduction and the numerical absorption corrections, the program X-RED 32 (Version 1.01, STOE & Cie GmbH, 2001) was used. The structures were solved by conventional Patterson methods and subsequent Fourier syntheses and refined by full-matrix least squares for F^2 using the SHELX program [17]. The positions of all non-hydrogen atoms were deduced from difference Fourier maps and refined anisotropically with the exceptions described below. In compound **1**, most of the partially occupied water molecules were refined isotropically and were either given a common U(eq) value to allow free refinement of the site

occupation factors, or the latter were fixed at appropriate values and the U(eq) values refined. Part of the acetone molecules, which are all fully occupied, as well as part of the second counter ion were only refined isotropically, as well as O134 of one molecule as it would turn into "non-positive refined". Hydrogen atoms were placed in calculated positions and refined with isotropic displacement parameters, while no hydrogen atoms were added to the acetone, the water molecules, as well as to the ribose O5R. The entire {enPtNCl} moiety in compound **2** is disordered over two positions with occupancy factors of 75% and 25%, respectively. Atoms $N1_{CN}$_A, N12_A, C11_A, and C12_A of the lower occupied {enPtNCl} moiety could only be refined isotropically or they would result in a "non-positive refined" state. One of the amide groups of the corrin ring, consisting of C83, N84, O84, is likewise disorderd over two different positions of 75% and 25% occupancy each. Again, the lower occupied group is kept isotropically. All partially occupied water molecules were refined isotropically with a common U(eq) value and the site occupation factors was left to refine freely. The counter ions is spread over two positions, again with occupancies of 75% and 25%, respectively, and was refined isotropically. One acetone molecule (60%, anisotropic refinement) shares its position with two water molecules (10%, and 40%), the other acetone (50%, isotropic refinement) is best described as sharing space with three water molecules (25% each). Hydrogen atoms were included in calculated positions and refined with isotropic displacement parameters. No hydrogen atoms were added to the 25% occupied {enPtNCl} moiety, the 25% occupied amide group, as well as to the acetone and water molecules. Crystallographic data and details of refinement are reported in Table 1.

3. Results and Discussion

*3.1 Characterization of [enPtCl-vitB_{12}]$CF_3CO_2 \cdot 15.8\ H_2O \cdot 1.1\ (CH_3)_2CO$ (**2**)*:

Complex **2** was prepared in analogy to the preparation of **1** (see also Scheme 1) [11]. The reaction of $enPtCl_2$ with one equivalent of silver nitrate led to the aqua species

$[enPtCl(H_2O)]^+$ as an intermediate, which was not isolated but subsequently reacted with vitamin B_{12} to give the $[enPtCl\text{-}vitB_{12}]^+$ adduct **2** in 89% yield.

The reaction between $[enPtCl(H_2O)]^+$ and vitamin B_{12} was followed by HPLC. After stirring of the reaction mixture at 50 °C for 17.5 h, the ratio between a new emerging peak at 7.7 min retention time and the peak of substrate vitamin B_{12} at 13.8 min was found to be around 80:20. Product **2** was isolated by preparative HPLC. The ESI-MS spectrum of $[enPtCl\text{-}vitB_{12}]^+$ (**2**) indicated a peak at m/z = 823.26 corresponding to the mass of the doubly charged product. This signal together with peaks of lower intensity at 822.27, 822.76, 823.77, 824.26 and 824.77 display the typical isotope pattern expected for the presence of one platinum atom. IR spectroscopy shows a clear shift of the ν_{CN} of **2** towards higher wavenumbers with respect to vitamin B_{12} (from 2134 to 2195 cm^{-1}), a typical finding caused by the electron-pulling effect of the platinum(II) coordination (Fig. 1) [18].

Based on earlier studies [19,20] and with the aid of [^{1}H,^{13}C]-HSQC and a [^{1}H,^{1}H]-COSY-NMR experiments, most ^{13}C-NMR and ^{1}H-NMR signals with shifts downfield from 3 ppm could be assigned. The most characteristic signals for B_{12}-derivatives are the 5 peaks between 7.3 and 6 ppm belonging to H2, H4 and H7 of the 5,6-dimethylbenzimidazole (DMB) moiety, H1R of the ribose as well as H10 of the corrin ring (Fig. 2a). These ^{1}H signals are very similar to the ones observed for *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^+$ (**1**) [11]. The ^{13}C-NMR signals of **2** are in good correlation with vitamin B_{12} [19] showing only small deviations between 0.02 and 0.62 ppm. The largest deviations are observed at C7, C8 and C72 of the corrin ring, at C4B, C8B and C9B of the 5,6-dimethylbenzimidazole, as well as at C2R of the sugar- and C175 of the aminopropanol moieties (Fig. 3a). Changes in chemical shift at the DMB ligand imply that the Pt^{2+} coordination influences the coordination properties of the cobalt center across the octahedron in the axial position. Chemical shifts assigned to ring B, i.e. of C7, C8, and C71, are probably influenced due to the positioning of the en-ligand above, which might be fixed also in solution by a hydrogen bond of one ammine proton at N12 to O73 located at ring B, as

observed in the crystal structure (see also Fig. 4a). The two carbon atoms of the ethylenediamine ligand gave signals at around 48 ppm and showed a strong correlation to a triplet at 2.36 ppm in the [^{1}H,^{13}C]-HSQC arising from the corresponding ethylene protons.

*3.2 Crystal structures of cis-[$(NH_3)_2$PtCl-vitB_{12}]CF_3CO_2 · 12.4 H_2O · 2.5 $(CH_3)_2CO$ (**1**) and [enPtCl-vitB_{12}]CF_3CO_2 · 15.8 H_2O · 1.1 $(CH_3)_2CO$ (**2**)*:

The crystal structure of compound **1** has been solved previously, but lacked one of the two $CF_3CO_2^-$ anions most likely due to disorder [11]. We now obtained crystals of both, **1** and **2**, by vapor diffusion of acetone into an aqueous solution of the compounds and were able to improve the structure of compound **1** in such a way, that both anions plus additional acetone and water molecules could be refined.

Complex **1** crystallizes in space group *P*1 with two complex molecules per asymmetric unit. Compound **2** (monoclinic *C*2) shows only one platinum complex in the asymmetric unit, but the axial CN-enPtCl unit is distributed over two positions with occupancies of 75 and 25 % (see also Tables 1 and 2 as well as Fig. 3b).

Overall, the two complexes **1** and **2** are very similar and the coordination geometries of the cobalt centers are virtually identical. The lengths of the axial bonds to the cyanide and the benzimidazole ligands in **1** and **2** also correspond quite well (Table 2) to the ones in free vitamin B_{12}: The Co-C_{CN} distances in **1**, **2** and vitB_{12} are identical within their error limits (1.858(12) Å in vitB_{12} [21]) as it is also true for the Co-N3B bond (2.011(10) Å in vitB_{12} [21]).

The platinum(II) centers show an almost perfect square planar geometry. Interestingly, the smallest Pt-N distance is in all cases the one to the cyanide ligand, although this is the bond most easiest broken, as the occasional occurrence of free vitB_{12} in the HPLC experiments shows (see also Section 3.4). In addition, it should be mentioned that both, the *cis*-$[(NH_3)_2PtCl]^+$ and the $[enPtCl]^+$ moieties, are located accordingly above the corrin plane,

being "fixed" by an intramolecular hydrogen bond between one am(m)ine proton and O73 of the B_{12} unit (Fig. 4a).

*3.3 "Base-on/base-off" behavior of **1** and **2**:*

The "base-on/base-off" behavior of coenzyme B_{12}, i.e. the intramolecular equilibrium of the coordination or release of the DMB moiety from the axial position at the cobalt center is of great biological importance. Coenzyme B_{12} exists predominately in the "base-on" form with the dimethylbenzimidazole moiety coordinated via N3 to the cobalt atom. However, recent crystallographic studies have shown that coenzyme B_{12} and its analogs can switch under certain conditions from the "base-on" to a "base-off/His-on" form when binding to their receptor protein by replacing the DMB ligand with the imidazole unit of a proteinogenic histidine residue [22-24].

In the absence of protein, the state of the intramolecular "base-on/base-off" equilibrium is dependent on the pH value of the solution. Upon acidification, protonation of the N3 position of the DMB ligands occurs causing the predominance of the "base-off" form at acidic pH [25]. This conformational change can be followed either by circular dichroism (CD) or by UV/Vis spectroscopy due to a change of the absorbance maxima from around 520 nm in the "base-on" form to around 460 in the "base-off" form [26-29]. AdoCbl has a $pK_{base\text{-}off} = 3.67$ [30] which is much more basic compared to vitamin B_{12}, which has a $pK_{base\text{-}off} = 0.1$ [31]. This difference may be explained by the much stronger electron donating effect of the ribose coordination compared to the anionic cyanide ligand. It is obvious, that the ease with which the DMB ligand is replaced by another coordinating moiety plays an important role in terms of binding capacity to proteins and other biomolecular complexes.

We investigated the "base-on/base-off" behavior of **1** and **2** by titrating a 50 µM solutions of each vitamin B_{12} derivative with HNO_3 in the pH range from 10 to –0.3 (Fig. 5). Nearly no changes in the UV/Vis spectra were found between pH 10 and 2 and a clear dominance of the

"base-on" form was found down to pH 1 in both cases. The spectrum of **2** still shows an excess of the "base-on" form at pH 0.5. As can be seen in Fig. 5, at pH 0, the characteristic "base-on" bands around 514 and 550 nm get broader and consequently lower in absorption. At pH values around –0.3 increasing amounts of the "base-off" form are present as can be seen from the emerging new maximum at around 500 nm. Compound **1** already starts to change to the "base-off" form at pH 0.2. Therefore, the p$K_{\text{base-off}}$ of the two B_{12} derivatives **1** and **2** are comparable to the one of vitamin B_{12}. This is not surprising as the coordination of the platinum(II) center to the cyanide ligand will not increase the electron density on the cobalt center but instead rather diminish it.

*3.4 Characterization of cis-$[(NH_3)_2Pt(9\text{-}MeAde)\text{-}vitB_{12}]^{2+}$ (**3**):*

In order to synthesize coenzyme B_{12} analogs, we added 9-methyladenine (9-MeAde) to the *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^+$ adduct (Scheme 1). As the superimposition of the structures of AdoCbl and complex **2** in Fig. 4b shows, the relative positions of the adenine N9 and the chloride ligand in **2** (which is replaced by a coordinating ring nitrogen of an adenine derivative) are in perfect agreement. The agreement will be still quite good when the longer Pt^{2+}-Cl bond (2.3 Å) is replaced by the shorter Pt^{2+}-N bond (typically 2.0 Å). Because of the corresponding positions of the adenine bases in the two compounds, it is well feasible that an adenine adduct of **1** or **2** consitutes a very good analogue of AdoCbl in terms of binding properties towards proteins, RNAs or other molecules.

It is well known that *cis*- and *trans*-platinum(II) centers can coordinate to all unsubstituted positions of nucleobase moieties with the N7 positions of purines and the N1 position of adenine being the predominant binding sites at neutral pH [32-40]. Indeed, it has recently been shown that **1** can coordinate to 9-methylguanine or 2'-deoxyguanosine through the N7 position under replacement of the second chloride-ligand [11]. In case of coordination to adenine derivatives the situation is a little more complicated as N1 and N7 compete for

binding [40]. Usually, N1 coordination can be suppressed to a large part by acidifying the reaction solution to a pH below 3 thereby blocking this site by protonation [41,42]. However, compounds **1** and **2** proved to be sensitive to acidic conditions as, e.g. storage overnight at 4 °C in 0.1% trifluoro acetic acid let to breakage of the cyanide-Pt bond in both complexes. Partial decomposition of the two complexes was also observed when **1** and **2** were treated with one equivalent of $AgNO_3$ to replace the chloro ligand with a water molecule in order to activate the Pt^{2+} center for coordination to the adenine moiety. Therefore, we reacted complex **1** with a large excess of 50 equivalents of 9-methyladenine in water without addition of any acid. The reaction was followed by HPLC and after one week of stirring at 50 °C, two new peaks between 5 and 7 min retention time (next to the peaks of the reagent and of free vitamin B_{12} deriving from decomposition) were detected. Compound **3** was isolated from the main peak at 6.5 min. Characterization of this product by analytical HPLC showed only one peak. In addition, analysis of **3** by LC-MS gave one single peak at m/z = 865.1, which corresponds to the mass of the twofold charged product *cis*-$[(NH_3)_2Pt(9\text{-MeAde})\text{-vit}B_{12}]^{2+}$ (**3**) lacking four hydrogen atoms. Taken together this is a clear indication that indeed the chloride ligand at the *Cisplatin* entity is replaced by 9-methyladenine, yielding the single product **3**.

A ^{1}H-NMR of **3** shows the aromatic protons of the 9-methyladenine moiety at 8.37 ppm and 8.21 ppm, which is further highfield compared to *cis*-$[(NH_3)_2Pt(9\text{-MeAde})Cl]^+$ (8.35 ppm for H2 and 8.57 ppm for H8) [42] and further downfield to free 9-MeAde (8.15 ppm for H2 and 8.00 ppm for H8) [42]. The strong downfield shift confirms the *Cisplatin* bridged species **3**. The same is true for the resonance of the C9-H_3 methyl group (see also Experimental Section). All signals assigned to the 9-methyladenine ligand of **3** are split in about a 1:1 ratio (Fig. 2b) indicating that not one but two species are present. One possibility could be that there is a hindered rotation around the Co-CN-Pt axis thus "fixing" the adenine ligand in two different positions and giving two conformational isomers. However, this seems unlikely because the 9-MeAde lies about 5 Å above the corrin plane, i.e. a distance which is by far

larger than stacking distances of typically around 3.3 – 3.4 Å. In addition, in case of the 9-ethylguanine adduct also no splitting of the aromatic H8 signal was observed [11]. A much more likely explanation – and the one we favor – is the presence of the N1 and N7 coordinated isomers, whose formation one would actually expect based on the reaction conditions in the absence of acid. Comparison with the chemical shifts of free 9-MeAde and its *Cisplatin* complex mentioned above, implies that the two aromatic signals more downfield at about 8.47 ppm belong to H8 and H2 of the N7 and N1 platinated species, respectively, as Pt^{2+} induces a strong downfield shift due to its electron pulling properties. Consequently, the signals at around 8.32 ppm belong to H2 of the N7-platinated and H8 of the N1-platinated species **3**. Unfortunately, a separation of such two isomeric forms was not successful so far due to their very similar properties. Nevertheless, we detected no decomposition of the *cis*-$[(NH_3)_2Pt(9\text{-MeAde})\text{-vitB}_{12}]^{2+}$ (**3**) complex in the NMR tube over several days. Considering that after the initial isolation of **3**, this complex was always handled under normal daylight conditions, this fact demonstrates the great potential of such more light stable cobalamin derivatives.

3.5 Summary and conclusion

Here we have presented a synthetic pathway towards the synthesis of light stable derivatives of adenosyl cobalamin with an adenine moiety covalently attached above the corrin plane. The intermediates *cis*-$[(NH_3)_2PtCl\text{-vitB}_{12}]CF_3CO_2 \cdot 12.4\ H_2O \cdot 2.5\ (CH_3)_2CO$ (**1**) and $[enPtCl\text{-vitB}_{12}]CF_3CO_2 \cdot 15.8\ H_2O \cdot 1.1\ (CH_3)_2CO$ (**2**) have been characterized by X-ray crystallography, ^1H- and ^{13}C-NMR spectroscopy, as well as by their intramolecular acid-base dependent "base-on/base-off" equilibria examined with UV/Vis spectroscopy. We showed that the coordination of the *cis*-$[(NH_3)_2PtCl]^+$ or $[enPtCl]^+$ entity to vitamin B_{12} has little influence on this equilibrium, which is also expected to be the case with compound **3**.

The 9-methyladenine adduct **3** could only be synthesized as a presumable mixture of two

isomeric forms, i.e. the N1 and N7 coordinated complexes. Nevertheless, to the best of our knowledge, this is the first time that such a light-stable adenosyl cobalamin derivative has been synthesized where the adenine base is not only directly linked to the cobalt-corrin system but should also have the same distance above the corrin plane as in AdoCbl. It should be noted however, that in AdoCbl, the adenine base is flipped, i.e. the N7 is in the position of the N9 position and *vice versa*, as N9 is bound to the ribose C1' atom (Fig. 4b). This means that together with the relative rigidity of **3**, a different hydrogen bonding pattern of this model compound is expected. Nevertheless, this differenc might not be crucial because the predominant interaction of the adenosine ligand when binding to a protein or a RNA riboswitch might very well be purelly based on stacking interactions with aromatic amino acid side chains or nucleobases, as the adenine base is known to have excellent stacking properties [43-46]. The correct distance of about 5 Å between the 9-methyladenine and the corrin ring in **3** ensures that stacking interactions with the host molecule would still be possible (Fig. 4b).

To summarize, compound **3** represents a model system for AdoCol to (i) investigate the replacement of the benzimidazole moiety by a host molecule side chain, and (ii) to characterize the importance of the stacking interaction of the adenine base with the host molecule.

Acknowledgements

We thank Prof. Dr. Roger Alberto, Pilar Sanchez, and Dr. Stefan Mundwiler for many helpful discussions as well as Dr. Serge Chesnov for recording the ESI-MS spectra and Nikos Agorastos for recording the LC-MS spectra. Financial support by the Swiss National Science Foundation (project funding to E.F, 21-105269/1 and a *SNF-Förderungsprofessur* to R.K.O.S., PP002-68733/1) and the *Forschungskredit* of the University of Zürich (to S.G.) is gratefully acknowledged.

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Table 1. Crystallographic data for compounds **1** and **2**.

	1	**2**
Formula	$C_{145}H_{267.6}Cl_2Co_2F_6N_{32}O_{61.8}P_2Pt_2$	$C_{70.3}H_{134.2}ClCoF_3N_{16}O_{32.9}PPt$
Formula weight	4203.19	2107.30
Crystal system	triclinic	monoclinic
Space group	*P*1	*C*2
a / Å	16.922(3)	33.832(7)
b / Å	17.298(3)	17.282(3)
c / Å	18.101(3)	16.995(3)
α / °	112.77(2)	90.00
β / °	98.47(2)	100.13(3)
γ / °	91.69(2)	90.00
V / Å^3	4810(1)	9782(3)
Z	1	4
D_{calc} / g cm^{-3}	1.451	1.431
μ(Mo Kα) / mm^{-1}	1.758	1.731
F(000)	2184	4372
Crystal size / mm	0.32 × 0.10 × 0.10	0.35 × 0.15 × 0.10
Temperature / K	183(2)	183(2)
Radiation / Å	Mo Kα (0.71069)	Mo Kα (0.71069)
θ_{min}, θ_{max} / °	2.30, 24.71	1.87, 25.81
Dataset	-19:19; -20:20; -21:21	-41:41; -21:21; -20:20
Tot., uniq. data, R_{int}	60944, 30817, 0.078	34604, 18674, 0.045
Observed data [$I > 2\ \sigma(I)$], N_{par}	27849, 1605	14824, 1177
R, w*R*2, *S* [$I > 2\sigma(I)$][a]	0.0564, 0.1421, 1.075	0.0482 0.1076, 0.980
Max. and av. shift/error	0.00, 0.00	0.00, 0.00
Min. and max. resd. dens. [e Å^{-3}]	2.81, -1.44	0.61, -1.14

[a] $R1 = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$, $wR2 = [\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2]^{1/2}$.

Table 2. Selected bond and hydrogen bond lengths (Å) as well as angles (°) for compounds **1** and **2**.

	1 (mol a)	**1** (mol b)	**2**[a]
Pt-Cl	2.305(3)	2.310(3)	2.318(3)
Pt-N11	2.041(8)	2.032(7)	2.036(6)
Pt-N12	2.061(9)	2.029(8)	2.026(8)
Pt-N_{CN}	1.977(8)	1.962(8)	1.960(8)
Co-C_{CN}	1.875(9)	1.849(8)	1.870(5)
Co-N3B	2.020(8)	2.046(7)	2.025(4)
N11-Pt-N_{CN}	177.7(4)	179.3(4)	176.8(5)
N12-Pt-Cl	177.6(3)	178.0(2)	177.2(2)
N3B-Co-C_{CN}	179.2(4)	179.3(4)	178.3(2)
N12···O73	2.852(12)	2.910(10)	2.872(8)

[a] The values given are for the atoms with 75% occupancy.

Figure Legends

Fig. 1. Section of the IR-spectra of vitamin B_{12} (top), *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^+$ (**1**) (bottom) and $[enPtCl\text{-}vitB_{12}]^+$ (**2**) (middle). The ν_{CN} bands of both Pt^{2+} adducts (**1**, 2199 cm^{-1}; **2**, 2195 cm^{-1}) are shifted towards higher wavenumbers compared to vitamin B_{12} (2134 cm^{-1}) being a clear indication for a bridging cyanide ligand [18].

Fig. 2. ^{1}H-NMR spectra of (a) $[enPtCl\text{-}vitB_{12}]^+$ (**2**) and (b) of the *cis*-$[(NH_3)_2Pt(9\text{-}MeA)\text{-}vitB_{12}]^{2+}$ adduct (**3**). Signals of the benzimidazole ligand (●), the ribose H1R (■), and H10 (▲) of the corrin ring are indicated. The three signals belonging to the 9-MeAde moiety are split (see insert in b), implying the presence of both N7 and N1 coordinated 9-methyladenine (see also text). The more downfield signals (◇) are assigned to H2 and H8 of the N1- and N7-platinated species, respectively, whereas the corresponding H8 and H2 further away from the respective platination site give resonances around 8.32 ppm (□). The methylgroup (○) of the 9-methyladenine ligand is also indicated.

Fig. 3. Numbering scheme and crystal structure of $[enPtCl\text{-}vitB_{12}]CF_3CO_2 \cdot 15.8\ H_2O \cdot 1.1\ (CH_3)_2CO$ (**2**). (a) Numbering scheme of $[enPtCl\text{-}vitB_{12}]^+$ as used in this study, corresponding to the one introduced in ref. [1]. (b) Ortep plot of **2** showing ellipsoids of 50% probability together with the atom numbering scheme.

Fig. 4. Superimposition of structures of B_{12} derivatives. (a) Overlap of the crystal structures of **1** and **2**, illustrating that these two complexes virtually have the same conformation. In both structures, the am(m)ine ligands form an intramolecular hydrogen bond to O73 of a corrin ring side chain of the $vitB_{12}$ entity. (b) Stereoview of the superimposition of the crystal structures of coenzyme B_{12} (grey, CCDC DADCBL) [47] and complex **2** (black). It can be well seen that the N9 position of the adenine base occupies the exact same position as the

chloride ligand in **2** therefore underlining the validity of the here presented model system. The positions of the chloride ligand (a, b) and the adenine N9 (b) are highlighted by black and grey spheres, respectively. The side chains at the corrin ring are omitted for clarity.

Fig. 5. Section of the UV/Vis spectra of [enPtCl-vitB_{12}]$^{+}$ (**2**) at different pH values ranging from pH 8 to –0.3 . The "base-on" form with a maximum at 550 nm is solely present down to pH 0.5. At pH values below zero the dimethylbenzimidazole moiety becomes protonated and the "base-off" form absorbing at 465 nm starts to gain importance. Titration points: pH 8, black line; pH 2, dark grey line; pH 1, grey line; pH 0.5, black dotted line; pH 0.2, dark grey dotted line; pH 0, grey dotted line; pH –0.3, light grey dotted line.

Scheme 1. Formation of the *cis*- or enPt-bridged vitamin B_{12} derivatives and consecutive reaction with 9-methyladenine to give a mixture of N7 and N1 coordinated adduct(**3**).

Synopsis:

Sofia Gallo, Eva Freisinger and Roland K. O. Sigel

Towards the synthesis of light stable coenzyme B_{12} analogs

The synthesis, structural characterization, as well as the pH dependent "base-on/base-off" behavior of cyanide bridged vitamin B_{12} conjugates with either a *cis*-$[(NH_3)_2PtCl]^+$ or a $[enPtCl]^+$ moiety are reported. Subsequent reaction of *cis*-$[(NH_3)_2PtCl\text{-}vitB_{12}]^+$ with 9-methyladenine led to the corresponding adenine adduct as a light stable AdoCbl analog.

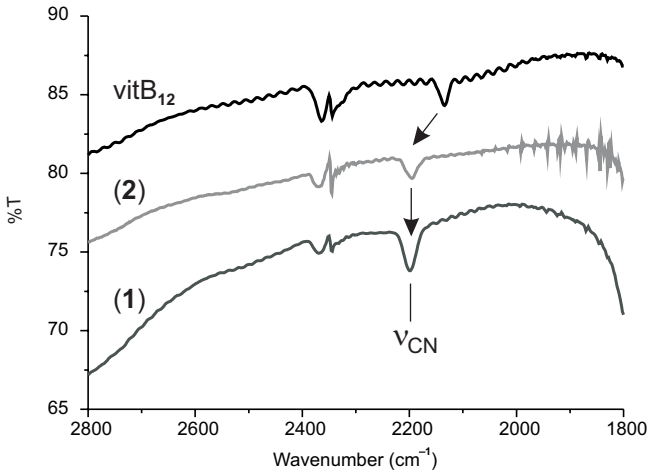

90
85
80
75
70
65
%T
vitB12
(2)
(1)
νCN
2800
2600
2400
2200
2000
1800
Wavenumber (cm−1)

Figure 1

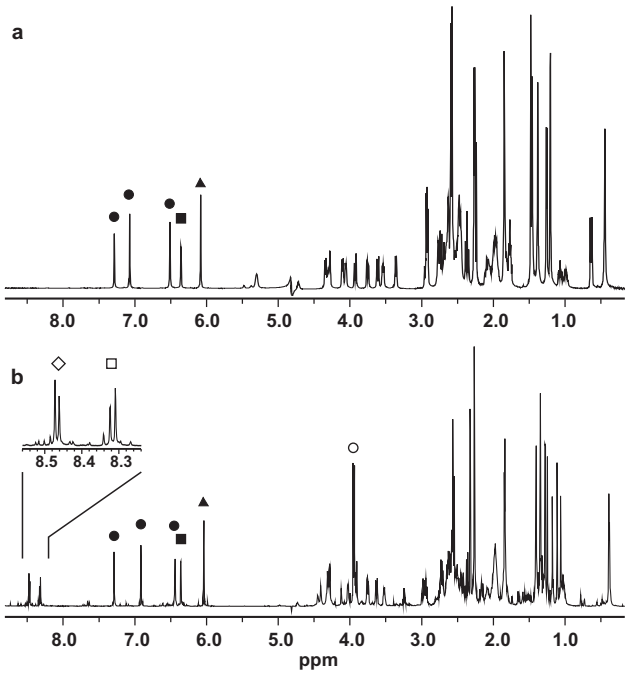


Figure 2

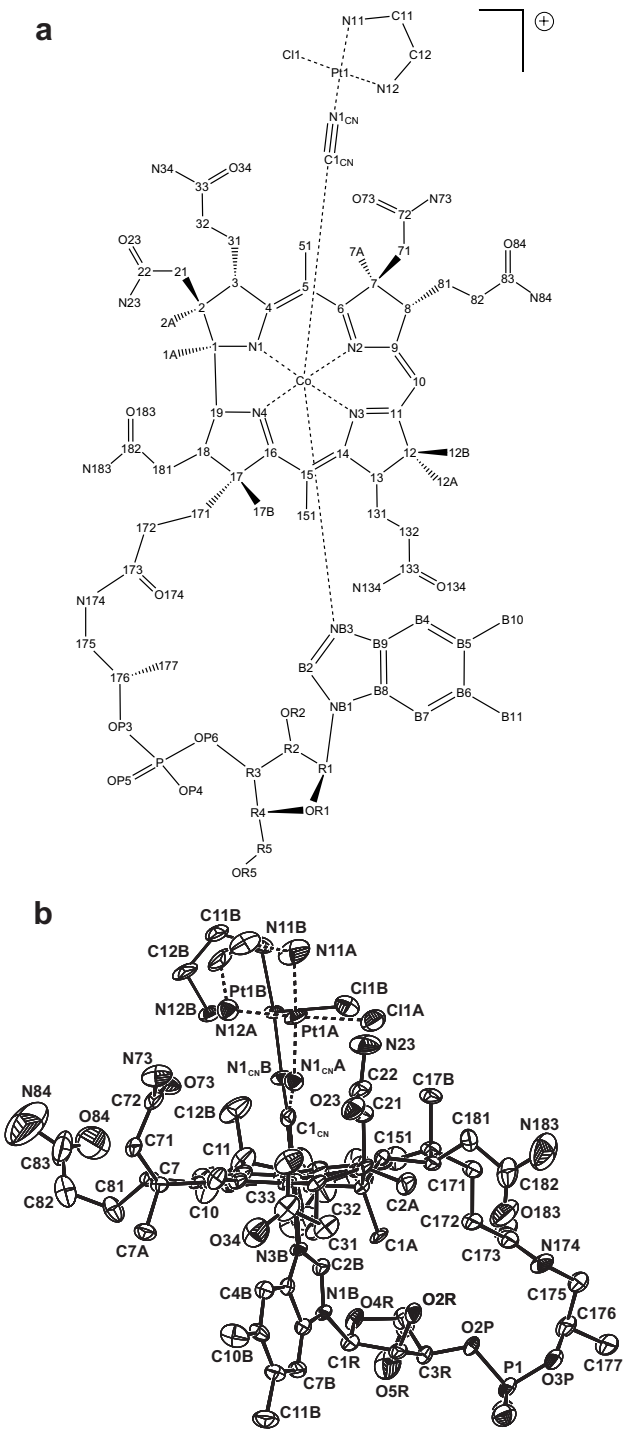


Figure 3

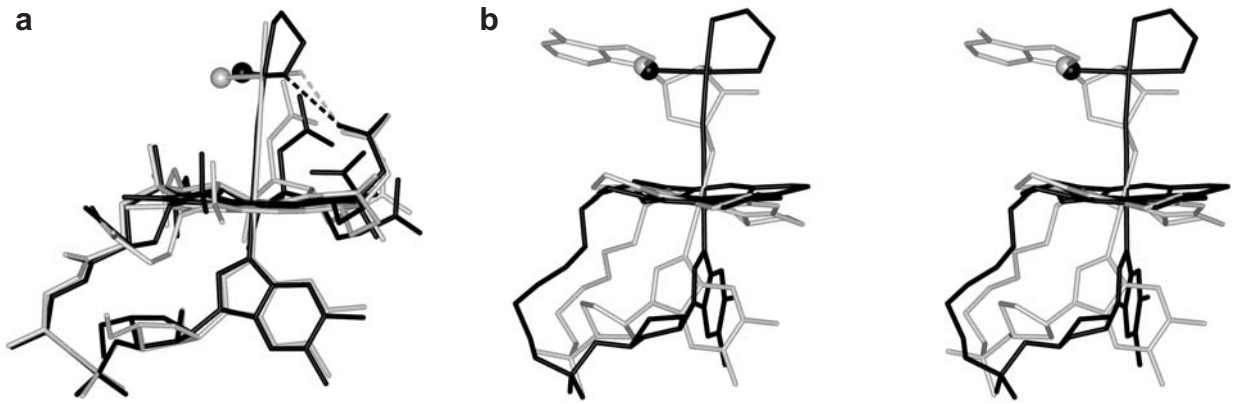


Figure 4

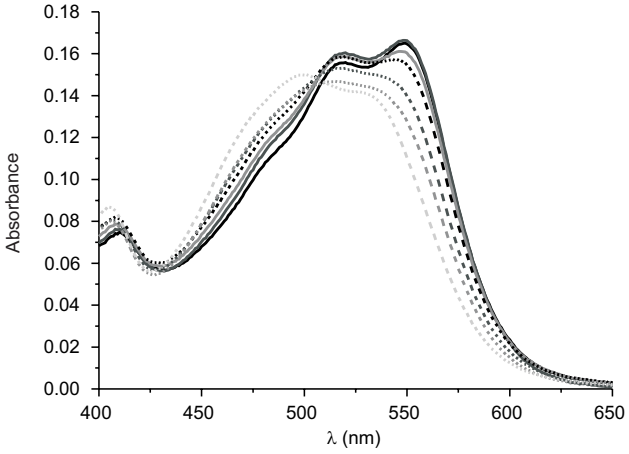
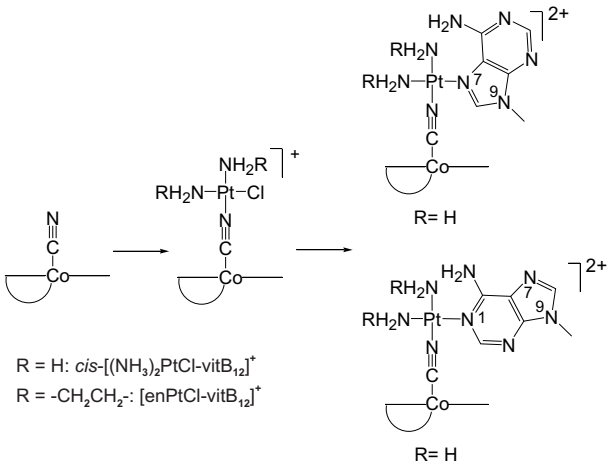
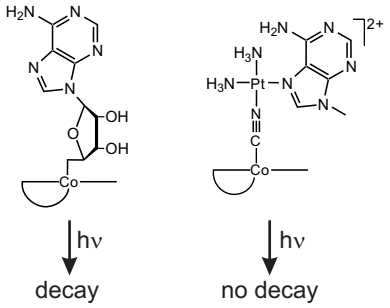

0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Absorbance
400
450
500
550
600
650
λ (nm)

Figure 5



Scheme 1



Graphical Abstract